Agglomeration of nano- and microplastic particles in seawater by autochthonous and de novo-produced sources of exopolymeric substances

Supplementary materials and methods.

Preparation of glassware

All glassware used for these experiments had been pre-baked at 550°C for two hours to burn off any plastic residues present inside the vessels. The vessels were further cleaned with 5% nitric acid, then acetone, rinsed with distilled water and autoclaved prior to use. All the vessel used for the generation of marine snow were amber in colour to limit light-induced effects.

Bacterial strain

The bacterial strain used in this study to generate EPS was *Halomonas* species TGOS-10 that had been isolated from the Gulf of Mexico during the Deepwater Horizon oil spill [1]. The bacterial strain was grown on a marine broth (ZM/10) composed of ¾-strength natural seawater, peptone (0.05%), yeast extract (0.01%), and supplemented after autoclaving with filter-sterile (0.2 μm) trace elements and vitamins to final concentrations as previously described [2].

Roller-bottle incubations

For this, we used a low-profile roller table (Stovall, North Carolina, USA) on which the bottles were maintained in constant rotary motion under *in-situ* natural seawater temperature, unless otherwise indicated. This method of generating artificial agglomerates also allowed overall control to maintain a constant motion that minimised shear stress which would hinder the generation, or cause the disintegration, of agglomerates. However, the limitation of shear stress within the cylinders has been reported to overestimate the size of agglomerates in long term studies [3]; therefore, where size was recorded, we restricted the duration of our experiments to 24 hours.

Estimations of spherical diameter

To measure the total number and area of plastic agglomerates that formed in these SSE incubations, agglomerates captured on the filters were visualised using a Leica DM IRE2 confocal fluorescence microscope. Images were captured and the abundance and area of the agglomerates was calculated by using the particle analysis feature within the Fiji image analysis software [4]. These area data were then converted to equivalent spherical diameter

(ESD) using the following formula, as transposed from the projected spherical diameter formula present by Jennings and Parslow [5]:

$$ESD = \sqrt{\frac{4a}{\pi}}$$

where a is the measured area of the agglomerates, and ESD is their equivalent spherical diameter.

Surface tension measurements

The fluorescent plastic particles used in this study were received from the manufacturer (Phosphorex Inc, MA, USA) as suspensions in deionised water and containing up to 0.1% tween 20. Since tween 20 is a surfactant, we determined if the addition of the plastics altered the surface tension of the natural seawater or synthetic seawater as it may have an influence on agglomerate formation in our experiments. In addition, the diameter and surface charge of the plastic used in these experiments was measured using a ZetaSizer (Nano-ZS, Malvern, UK).

Table S1. Characteristics of the plastics used for each of the NSE treatments

Treatment Type	Plastic size group	Plastic mass concentration ($\mu g\ mL^{-1}$)	Plastic particle concentration (particles per mL)	Supplier Catalogue number
NSE-1	None (neg control)	None (neg control)	None (neg control)	None (neg control)
NSE-2	50 nm	5	5.60×10^{10}	#2101,
NSE-3	1 μm	5	3.67×10^{6}	#2104
NSE-4	10 μm	5	8.15×10^{3}	#2106G

Supplementary references:

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3. Jackson, G. A., Coagulation in a rotating cylinder. *Limnology and Oceanography: Methods* **2015,** *13*, (4), 194-201.
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5. Jennings, B.; Parslow, K. In *Particle size measurement: the equivalent spherical diameter*, Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 1988, 1988; The Royal Society: 1988; pp 137-149.